Extreme rainfall and snowfall alter responses of soil respiration to nitrogen fertilization: a 3-year field experiment

Running head: Soil respiration under extreme precipitation

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Abstract

Extreme precipitation is predicted to be more frequent and intense accompanying global warming, and may have profound impacts on soil respiration (*Rs*) and its components, i.e., autotrophic (*Ra*) and heterotrophic (*Rh*) respiration. However, how natural extreme rainfall or snowfall events affect these fluxes are still lacking, especially under nitrogen (N) fertilization. In this study, extreme rainfall and snowfall events occurred during a 3-year field experiment, allowing us to examine their effects on the response of *Rs*, *Rh* and *Ra* to N supply. In normal rainfall years of 2011/2012 and 2012/2013, N fertilization significantly stimulated *Rs* by 23.9% and 10.9%, respectively. This stimulation was mainly due to the increase of *Ra* because of N-induced increase in plant biomass. In the record wet year of 2013/2014, however, *Rs* was independent on N supply because of the inhibition effect of the extreme rainfall event. Compared with those in other years, *Rh* and *Ra* were reduced by 36.8% and 59.1%, respectively, which were likely related to the anoxic stress on soil microbes and decreased photosynthates supply. Although N supply did not affect annual *Rh*, the response ratio (RR) of *Rh* flux to N fertilization decreased firstly during growing season, increased in nongrowing season and peaked during spring thaw in each year. Nongrowing season *Rs* and *Rh* contributed 5.5–16.4% to their annual fluxes, and were higher in 2012/2013 than other years due to the extreme snowfall inducing higher soil moisture during spring thaw. The RR of nongrowing season *Rs* and *Rh* decreased in years with extreme snowfall or rainfall compared to those in normal years. Overall, our results highlight the significant effects of extreme precipitation on responses of *Rs* and its components to N fertilization, which should be incorporated into models to improve the prediction of carbon-climate feedbacks.

Introduction

It is expected that extreme precipitation events will increase in both frequency and magnitude accompanied by climate warming (Fischer & Knutti, 2015; Donat *et al*., 2016). The intensified global hydrological cycle can have profound consequences on the terrestrial carbon (C) cycle and its feedbacks to climate change (Knapp *et al*., 2008; Frank *et al*., 2015). Soil respiration (*Rs*) is the largest C flux from terrestrial ecosystems to the atmosphere in the global C cycle with a range of 68–98 Pg C yr^{-1}, and exceeds carbon dioxide (CO_2) emissions from fossil fuel burning by an order of magnitude (Raich & Schlesinger, 1992; Raich *et al*. 2002; Bond-Lamberty & Thomson, 2010).

Increasing nitrogen (N) input to ecosystems is another global change factor. Although a large number of studies have been conducted, the effects of N enrichment on *Rs* still remain controversial, with increases, decreases, and no changes being reported (Janssens *et al*., 2010; Zhou *et al*., 2014; Zhong *et al*., 2016). Inconsistence of these results is partly because *Rs* is a multisource flux, mainly including the heterotrophic (*Rh*) and autotrophic respiration (*Ra*). *Rh* originates from the decomposition of soil organic matter (SOM) and plant residues, and is driven by the microbial activities and the quantity and quality of organic C substrates (Luo & Zhou, 2006; Trumbore, 2006). *Ra* integrates the respiration of roots and rhizosphere microbes and is closely related with belowground allocation of recently assimilated photosynthates (Högberg *et al*., 2001; Wan & Luo, 2003; Tang *et al*., 2005). N addition may increase soil microbial biomass and activity (Allison *et al*., 2010) or decrease enzyme activity and SOM decomposability (Treseder, 2008), resulting in corresponding changes of *Rh*. Likewise, N application may stimulate *Ra* due to increased plant growth (Cleveland & Townsend, 2006) or

suppress it by reducing belowground C allocation (Kuzyakov *et al*., 2002; Wang *et al*., 2017). On the other hand, *Rs* is regulated by numerous environmental factors, mainly including soil temperature and moisture, which can interact with N supply in affecting *Rs*. Xu & Wan (2008) showed that the stimulation effect of N fertilization on *Rs* was greater in a year with more precipitation. While, Yan *et al*. (2010) reported that N addition increased *Rs* in a wet year, but decreased it in a dry year. Different sensitivities of *Rh* and *Ra* to changing soil moisture complicate the effects of N fertilization on *Rs*. It has been suggested that *Rh* responded more rapidly to the variation in soil moisture than *Ra* because larger amount of moisture are needed to trigger the activities of plant roots than the shallowly located soil microbial communities (Parton *et al*., 2012). However, in a grassland ecosystem, *Ra* declined more greatly with decreasing soil moisture content than *Rh* (Gomez-Casanovas *et al*., 2012). Based on the collected global dataset, Chen *et al*. (2014) showed that *Ra* was related more closely with precipitation than *Rh*.

A number of manipulation experiments have examined the effects of increased precipitation on *Rs*. Recently, Liu *et al*. (2016) synthesized these results and found that increased precipitation stimulated *Rs*, *Rh* and *Ra*. However, cautions should be taken when predicting the trends of *Rs* under future precipitation scenario. To date, the number of precipitation manipulation studies separating *Rs* components is very limited (Liu *et al*., 2016). Moreover, these studies simulated increased rainfall amount through water addition, which may not necessarily reflect the real conditions of natural extreme rainfall events that may exceed the past and current variation range (Beier *et al*., 2012; Kayler *et al*., 2015). It is very likely that the extreme rainfall events could lead to soil water saturation or even being flooded

92 (Knapp *et al*., 2008). As a result, the limited oxygen (O_2) condition is expected to suppress the
93 activities of soil microbes and plant roots and then lower *Rh* and *Ra* (Rich & Watt, 2013;
94 Philben *et al.*, 2015). Despite the critical influences of extreme rainfall events on *Rs*, its
95 magnitude and direction is unclear under real-world condition. To the best of our knowledge,
96 no studies have investigated the effects of N fertilization on *Rs* and its components under
97 extreme precipitation conditions.

98 In the majority of previous studies, *Rs* is only measured during the warm or growing
99 season assuming that soil microbial activity is extremely low at low temperature (Fahnestock
100 *et al*., 1999; Hirano, 2005). However, it has been demonstrated that soil microbes can remain
101 active in cold environments, even at −39 °C (Panikov *et al*., 2006). The contribution of cold
102 season *Rs* to annual budget has been reported to be higher than 10% in Arctic tundra
103 (Fahnestock *et al*., 1999), temperate mountain forest (Mo *et al*., 2005) and Tibetan alpine
104 grassland (Wang *et al*., 2014). Particularly, peak CO_2 fluxes can be observed during the spring
105 thaw period, which are probably sourced from the physical release of CO_2 trapped below the
106 frozen horizons (Elberling & Brandt, 2003) or microbial decomposition as a result of
107 increased temperature, moisture or substrates (Hirano, 2005; Monson *et al*., 2006; Wang *et al*.,
108 2013). Grogan & Jonasson (2006) proposed that the seasonal maximum snow depth was a
109 primary determinant of wintertime CO_2 releases in a Swedish subarctic region. More snowfall
110 can induce higher CO_2 fluxes as a result of higher soil temperature under the deeper snow
111 cover (Björkman *et al*., 2010) or higher moisture content after snow melting (Wu *et al*., 2010).
112 However, Schindlbacher *et al*. (2014) reported that snowfall played a rather marginal role on
113 soil CO_2 emissions from a mid-elevation temperate forest. It is predicted that although future

climate warming will cause a decrease in mean snowfall, the frequency of snowfall extremes will be higher in many Northern Hemisphere regions (O'Gorman, 2014). Therefore, the impacts of snowfall on *Rs* and its response to N input need to be better understood to improve the predictions of *Rs* under future climate change.

The present study investigated the response of *Rs* and its components to N fertilization under extreme rainfall and snowfall events. We measured *Rs* and separated *Rh* and *Ra* with the root exclusion method in a temperate cropland during both growing and nongrowing seasons for three years. Our objectives were to (1) examine the effects of N fertilization on *Rs* and its components, (2) evaluate the impacts of extreme rainfall on the responses of *Rs* and its components to N fertilization, and (3) quantify the magnitude of nongrowing season respiration and its responses to N fertilization under extreme precipitation conditions.

Material and methods

Site description

The experiment was carried out in a rainfed maize-cultivated cropland at the Hailun National Agro-ecological Experimental Station, Heilongjiang Province, China (47°26'N, 126°38'E). The study region has a temperate continental monsoon climate. From 1953 to 2013, the mean annual air temperature was 1.9 °C (Fig. 1) with the monthly average ranging from −21.6 °C in January to 21.6 °C in July. Mean annual precipitation is 556 mm, with 87% falling during the crop growing season from May to October. The long-term climate data were sourced from the National Meteorological Information Center of China (http://cdc.nmic.cn/home.do).

Soil at the study site is characterized by a clay loam texture with 8% sand, 72% silt and 20%

clay, and classified as black soil or Typic Hapludoll based on genetic classification and the USDA soil taxonomy, respectively. The soil (0–20 cm) prior to the experiment had a pH of 5.9, bulk density of 1.0 g cm^{-3}, and contained 27.5 g kg^{-1} organic C, 2.2 g kg^{-1} total N, 6.7 mg N kg^{-1} ammonium (NH_4^+), 30.7 mg N kg^{-1} nitrate (NO_3^-) and 33.7 mg kg^{-1} dissolved organic C (DOC). Soil texture was determined by a laser particle size analyzer (LS13320, Beckman Coulter, Brea, USA). Soil pH was measured in a 1:2.5 soil-water ratio. Bulk density was determined with the intact core method. The soil organic C and total N contents were analyzed using the wet oxidation redox titration and micro-Kjeldahl method, respectively, and the mineral N and DOC concentrations were measured as described below.

Experiment design

The field experiment was conducted throughout three consecutive growing and nongrowing seasons, i.e., from 13 May 2011 to 10 May 2012, from 17 May 2012 to 14 May 2013 and from 19 May 2013 to 24 April 2014. Two treatments were included in this study: a N-fertilized treatment (NF) and the control without N application (CK). In May 2011, six plots of 12 × 4.2 m^2 were established with three replicates for each treatment. In the other two years, four replicates were arranged to reduce the spatial variation and the plots were set in 4 × 4.2 m^2. N fertilizer was applied as urea at the rate of 150, 200, and 150 kg N ha^{-1} in 2011/2012, 2012/2013 and 2013/2014, respectively. Urea application was split between pre-plant and sidedress with a ratio of 1:1. The pre-plant fertilization occurred on 13 May 2011, 16 May 2012 and 19 May 2013. Sidedress urea was applied at the maize V6–V8 growth stage on 15 June 2011, 27 June 2012 and 28 June 2013.

158 As a local practice, field is split into ridges and furrows at a distance of 70 cm. After the
159 pre-plant fertilizer banded in the ridges, maize seeds were immediately sown in a plant
160 spacing of 25 cm. The pre-emergent herbicide was used for weed control and visible weeds
161 were removed by hand throughout the experiment. Mature maize was harvested on 27
162 September 2011, 2 October 2012 and 5 October 2013. All the crop residues were removed
163 from the plots followed by manual tillage. Samples of maize grain and straw were oven-dried
164 at 60 °C until constant weight to obtain the grain yield and aboveground biomass.
165
166 *Soil respiration measurement*
167 Soil CO_2 fluxes were measured over a 3-year period with the static closed chamber method. A
168 root exclusion method was used to partition *Rs* into *Rh* and *Ra*. In each plot, a cylindrical
169 polyvinyl chloride (PVC) tube was inserted 5 cm into soil at the center of one ridge, including
170 one maize plant. A PVC base frame (70 cm width, 30 cm length and 20 cm height) was
171 pushed into soil at a depth of 10 cm, making the tube located at its center. The PVC tube and
172 base frame were fixed before sowing and permanently during each year's measurement period.
173 When taking gas samples, a PVC pipe was embedded to the tube. A stainless steel chamber
174 (70 cm width, 25 cm length and 20 cm height) insulated with white foam was fitted to the
175 base frame. The chamber was custom-made, consisting of two separate parts that were
176 connected by two hinges and sealed with rubber strips. In the middle of the chamber top, an
177 opening was made to fit the pipe with sealing trips to ensure airtightness. An area of 1.4 × 2.1
178 m^2 in each plot was left unplanted. The base frame was inserted 20 cm into soil at the center
179 of this area. A PVC chamber was used for gas sampling. See Ding *et al.* (2007) for further

detailed information of the devices.

Gas samples were taken twice per week during the growing season and spring thaw period, weekly after harvest until soil freezing, and biweekly during the frozen period, resulting in 69, 65 and 71 times in 2011/2012, 2012/2013 and 2013/2014, respectively. Gas samples were collected between 09:00 and 12:00 hours to minimize diurnal variation in the flux pattern. Each time, four chamber headspace air samples were drawn using a plastic syringe at 0, 10, 20 and 30 min after chamber closure and injected into pre-evacuated 20 mL glass vials. Meanwhile, the chamber air temperature was recorded. Gas CO_2 concentration was analyzed on a gas chromatograph equipped with a flame ionization detector (Agilent 7890, Santa Clara, USA). The chamber CO_2 concentration generally increased linearly during the closure time. Thus, the CO_2 flux rate was calculated from the slope of the linear regression between time and CO_2 concentration. Data were discarded if the R^2 of the linear fitting was below 0.85. The cumulative emission was calculated by linear interpolation between measurement days.

The *Ra* is calculated as the difference between *Rs* and *Rh* measured in the planted and unplanted area, respectively, after seedling emergence until maize roots were removed from soil. It should be noted that the root exclusion method has some known drawbacks. Carbon input from the root exudates and turnover may have priming effect on the decomposition of bulk SOM and affect the heterotrophic soil microorganisms (Kuzyakov & Gavrichkova, 2010). However, Kuzyakov & Cheng (2004) reported that the rhizosphere priming effect on *Rh* was very minor in a maize-planted soil. In the present study, to avoid long term bias caused by the lack of organic matter input from plant, a new area in the same field was used to establish the experimental plots in each year. Another possible bias is that root exclusion may change soil

202 temperature and moisture conditions (Subke *et al*., 2006). Our previous experiments in the
203 same study region showed that there were no significant differences of soil temperature and
204 moisture between the planted and unplanted plots during the maize growing season (Ni *et al*.,
205 2012). Suleau *et al*. (2011) pointed out that compared with forest and grassland ecosystems,
206 the root exclusion method was more effective and introduced less biases in cropland. A
207 trenching experiment evaluating the effect of root exclusion on soil microbial biomass and
208 community structure will help to assess the uncertainty in examining the response of *Rs* and
209 its components to N fertilization using this method. Indeed, Gavrichkova & Kuzyakov (2008)
210 reported that responses of maize root respiration to N fertilization evaluated by ^{14}C labeling
211 and comparison of planted and unplanted soils were similar.
212
213 *Auxiliary measurement*
214 Precipitation, air temperature, photosynthetically active radiation (PAR) and the depth of
215 snow cover and soil frost were obtained from a meteorological station. PAR data were lack in
216 August 2013 due to instrument failure after the extreme rainfall event. During the non-frozen
217 period, soil moisture content was determined in each plot with a TDR probe simultaneously
218 with gas measurement and expressed as water-filled pore space (WFPS). Soil temperature was
219 measured with a digital thermometer or geothermometer (in winter) at 5 cm depth. After
220 fertilization until soil freezing, soil mineral N concentrations were measured weekly in each
221 year, soil DOC concentrations were measured biweekly in 2012/2013 and weekly in
222 2013/2014. In addition, soil mineral N and DOC were detected at the end of each year's
223 experiment and weekly after soil thaw in 2014. Field-moist soil samples were extracted with 2

224 M KCl solution (shaken for 1 h and filtered through Whatman #42 filter paper), and the NH_4^+
225 and NO_3^- concentrations were analyzed on a continuous-flow autoanalyzer (San++, Breda,
226 the Netherlands). Soil DOC was extracted with deionized water (shaken for 1 h, centrifuged
227 for 10 min at 10000 rpm and filtered through 0.45 μm polyethersulfone membrane) and
228 measured on a TOC analyzer (vario TOC Cube, Elementar, Hanau, Germany).
229
230 *Data analysis and statistics*
231 Before statistical analysis, data normality was tested using the Kolmogorov-Smirnov test, and
232 square root or natural-log transformation was used as necessary. Paired t-test was performed
233 to evaluate the effects of N fertilization on respiration and plant biomass. The interannual
234 differences were analyzed with one-way analysis of variance (ANOVA) with the least
235 significant difference test for multiple comparisons at $P < 0.05$. Repeated-measures ANOVA
236 was used to examine the effects of sampling time and treatment on the fluxes of *Rs*, *Rh* and
237 *Ra*, and soil NH_4^+, NO_3^- and DOC concentrations. Pearson analysis was conducted to probe
238 the correlations between CO_2 flux and environmental factors. The temperature sensitivity of
239 *Rs*, *Rh* and *Ra* were assessed by exponential function and the Q_{10} value (the change in CO_2
240 flux with a 10 °C increase in soil temperature at 5 cm). A forward stepwise multiple
241 regression analysis was used to explore the key factors among PAR, soil temperature,
242 moisture, NH_4^+ and NO_3^- that controlled *Rs*, *Rh* or *Ra*. Linear or exponential regression
243 models were developed to evaluate the relationships between *Rs*, *Rh* or *Ra* with soil or plant
244 parameters. A response ratio (RR) of *Rs*, *Rh* or *Ra* to N fertilization was calculated as the
245 ln-ratio of the value in NF to CK. The temporal pattern of RR of *Rh* flux was described by a

246 cubic regression model in each year. All statistical analyses were conducted with SPSS 18.0
247 (SPSS Inc., Chicago, USA) or Origin Pro 8.5 (OriginLab, Northampton, USA).
248
249 **Results**
250 *Meteorological conditions*
251 The annual mean air temperature in each year did not deviate from the long-term average (Fig.
252 1a). Daily air temperature ranged from −31.7 °C on 8 January 2013 to 28.3 °C on 19 June
253 2011 during the 3-year experimental period (Fig. 2a). Photosynthetically active radiation
254 (PAR) showed large variation from day to day and followed the seasonal pattern of air
255 temperature except in summer 2013. Annual precipitation was similar in 2011/2012, but
256 higher in 2012/2013 and 2013/2014 than the long-term average (Table S1). A record-breaking
257 extreme rainfall event occurred on 30 July 2013 with a daily rainfall of 178 mm (Liu *et al*.,
258 2014), leading this year to be the wettest in the study region since record began in 1953 (Fig.
259 1a). An extreme snowfall event of 17 mm was recorded on 12 November 2012. Total snowfall
260 and the maximum snow depth were higher in 2012/2013 than 2011/2012 and 2013/2014
261 (Table S1). Winter (November to March) precipitation in 2012/2013 was the highest on record
262 (Fig. 1b). The highest water-filled pore space (WFPS) was detected after field was
263 waterlogged in early August 2013(Fig. 2b). After soil thaw, WFPS increased in each year,
264 especially in 2012/2013 with a value of up to 88%.
265
266 *Soil mineral N and DOC*
267 Soil mineral N concentrations increased after fertilization at first and then gradually declined

in the NF treatment but always maintained at lower levels in CK (Fig. S1). A significant increase in soil NH_4^+ concentration was found after the extreme rainfall in August 2013, but the NO_3^- was largely suppressed. Soil DOC varied less than mineral N, and tended to increase after soil waterlogging and thawing in 2013/2014. N fertilization did not affect soil NH_4^+ concentration in the first year, but significantly increased it in 2012/2013 ($P < 0.01$) and 2013/2014 ($P < 0.05$). The NF treatment exhibited higher NO_3^- level compared to CK in all three years ($P < 0.01$). The mean soil DOC concentration was increased by N fertilization in 2012/2013 ($P < 0.01$), but not in 2013/2014.

Soil respiration and its components

The flux rates of *Rs* and *Rh* during the growing season exhibited similar temporal dynamics in 2011/2012 and 2012/2013 for both control and N fertilization treatments (Fig. 2c and d). In these two years, *Rs* and *Rh* increased with soil temperature, reached a maximum of above 200 and 100 mg C m^{-2} h^{-1}, respectively in July or early August, and then gradually declined until maize harvest. In general, CO_2 fluxes increased after rainfall. However, the extreme rainfall event occurring on 30 July 2013 led to sharp declines of *Rs* and *Rh* to below 36 and 27 mg C m^{-2} h^{-1}, respectively, until 27 August 2013. The flux of *Ra* showed similar seasonal pattern to *Rs* and *Rh*, and were generally higher in NF than CK (Fig. S2). However, after the extreme rainfall event in August 2013, *Ra* reduced to less than 15 mg C m^{-2} h^{-1} in both treatments. During the nongrowing season, *Rs* and *Rh* decreased to no more than 15 mg C m^{-2} h^{-1} after soil freezing in each year, but increased with increasing soil temperature and moisture after surface soil thaw in spring, particularly in April 2013. Repeated-measures ANOVA analysis

showed significant ($P < 0.001$) effects of sampling time on the fluxes of *Rs*, *Rh* and *Ra*, but the N treatment effect was not significant (Table S2). No distinct temporal variation was detected for the response ratio (RR) of *Ra* flux (Fig. 3). However, the RR of *Rh* flux showed consistent variation pattern among the 3 years, i.e., gradually decreased to below zero at 40–60th day after experiment onset, then got positive during the nongrowing season and peaked during the spring thaw period, which could be well depicted by a cubic function in each year.

Annual *Rs* ranged from 186 to 407 g C m^{-2} across the 3 years for the two treatments (Fig. 4a). *Rs* in 2013/2014 was significantly lower than 2011/2012 and 2012/2013, with a reduction by 45.2% and 51.0% on average for CK and NF, respectively. N fertilization significantly ($P < 0.05$) increased *Rs* by 23.9% and 10.9% in 2011/2012 and 2012/2013, respectively. However, in 2013/2014, N fertilization did not affect *Rs*. There was no significant difference in *Rh* between CK and NF in each year, averaging at 166 and 164 g C m^{-2} across the 3 years, respectively (Fig. 4b). Compared with the first two years, *Rh* in 2013/2014 was reduced by 39.1% and 34.6% for CK and NF, respectively. *Ra* was more greatly decreased in 2013/2014 than *Rh*, with a reduction of 53.3% and 64.9% for CK and NF, respectively (Fig. 4c). *Ra* in the NF treatment was significantly higher than CK in 2011/2012 and 2012/2013, but not in 2013/2014. The average contribution of annual *Rh* to *Rs* in the CK treatment was 59.0% (Fig. 4d) and significantly decreased to 53.0% by N fertilization. However, the ratio of *Rh*/*Rs* in 2013/2014 was similar between CK and NF, and was higher than the other two years.

Nongrowing season cumulative *Rs* and *Rh* were higher in 2012/2013 in comparison with the other two years with an average increase of 28.7% and 26.0%, respectively (Fig. 5). Nongrowing season *Rs* and *Rh* were significantly ($P < 0.05$) enhanced by N fertilization in all

three years. Particularly, in 2011/2012 *Rs* and *Rh* were 57.2% and 40.2% greater in NF than CK, respectively, and the RR of *Rs* and *Rh* were significantly ($P < 0.01$) higher than those in 2012/2013 and 2013/2014. The contribution of nongrowing season *Rs* and *Rh* to the annual budget ranged at 5.5–13.8% and 8.2–16.4%, respectively, across the 3 years.

Abiotic and biotic factors controlling soil respiration and its components

Correlations between soil temperature and *Rs*, *Rh* or *Ra* fluxes were significant in all 3 years except *Ra* for CK in 2013/2014 (Table S3–5). Generally, the Q_{10} value of *Rs* was higher in CK than NF as a result of decreased Q_{10} for *Rh* by N fertilization (Table S6). The Q_{10} values were lower in 2013/2014 than the other years, especially for *Ra*. Both *Rs* and *Rh* had higher Q_{10} values during the nongrowing season of 2012/2013 and 2013/2014 than the corresponding values in growing season of these two years and nongrowing season of 2011/2012.

There were significant correlations between total precipitation over 3 days prior to CO_2 measurement and *Rs* or *Rh* in 2011/2012 and 2012/2013 ($P < 0.01$), but not in 2013/2014. Correlation of *Ra* with precipitation was weaker, only significant in 2011/2012. Soil WFPS and *Rs*, *Rh* or *Ra* significantly correlated in 2011/2012, but not in the other years. For the growing season, linear models could describe the effects of soil moisture on *Rs*, *Rh* and *Ra* in 2011/2012 and *Rh* in 2012/2013 (Fig. 6). While, in 2013/2014 Gaussian models fitted well, with an optimal WFPS of 60%, 57% and 60% for *Rs*, *Rh* and *Ra*, respectively.

Ra was more correlated with PAR, especially with PAR_3 (average over 3 days prior to CO_2 measurement) than *Rs* and *Rh*, except in 2013/2014. This was corroborated by the stepwise regression analysis which showed that soil temperature and moisture were the key factors

driving *Rh*, while *Ra* was primarily regulated by PAR_3 instead of PAR (Table 1).

In general, *Rs*, *Rh* and *Ra* were more closely correlated with soil NO_3^- than NH_4^+. Soil DOC exhibited significant ($P < 0.05$) correlations with *Rs*, *Rh* and *Ra* with the combined data in 2012/2013 and 2013/2014. Consistently, annual *Rs*, *Rh* or *Ra* increased linearly with the annual mean concentration of soil NO_3^- or DOC, but not with NH_4^+ (Fig. 7). The aboveground biomass was significantly related with *Ra* ($P < 0.05$), but not with *Rs* and *Rh*.

Discussion

Effects of extreme rainfall on soil respiration and its components

A synthesis of precipitation manipulation studies suggested that soil respiration (*Rs*) would increase under future scenarios with increased precipitation (Liu *et al*., 2016). However, we found that annual *Rs* under control (CK) and N fertilization (NF) treatments in the wettest year of 2013/2014 was 45.2% and 51.0%, respectively, lower than the average in the other two years (Fig. 4a). To examine the effects of rainfall on *Rs* in the same region, we collected the published *Rs* measured in cultivated black soils during the maize growing season in normal rainfall years (Li *et al*., 2009; Liang *et al*., 2012; Ni *et al*., 2012; Li *et al*., 2013). After combining these data with our results measured in 2011/2012 and 2012/2013, we found that *Rs* from both unfertilized and N-fertilized soils increased linearly with total rainfall (Fig. 8a), which was in line with Liu *et al*. (2016). In contrast, *Rs* measured in 2013/2014 for both CK and NF treatments were greatly lower than the values calculated based on the linear functions obtained from the normal rainfall years. The decreased *Rs* largely resulted from the inhibition effects of extreme rainfall on each respiration component. Annual *Rh* in 2013/2014 was 39.1%

and 34.6% lower than the averages in the other two years for CK and NF, respectively (Fig. 4b). *Ra* was more strongly suppressed, with a reduction of 53.3% and 64.9% for CK and NF, respectively (Fig. 4c).

In 2011/2012 and 2012/2013, *Rh* was generally promoted by rainfall and increased with increasing soil moisture. Similar phenomenon has been widely reported in previous studies (e. g. Lee *et al.*, 2004; Matteucci *et al.*, 2015). Increased *Rh* after rainfall is mainly due to the enhanced microbial decomposition as a result of improved diffusion and availability of labile organic substrates, and increased microbial biomass and activity (Lee *et al.*, 2004; Borken & Matzner, 2009; Moyano *et al.*, 2013). In 2013/2014, however, *Rh* was not correlated to rainfall, and decreased if soil moisture exceeded the threshold of 57% WFPS. Specifically, the extreme rainfall event occurred on 30 July 2013 led soil to near saturation, and reduced *Rh* to 6.0–27 mg C m^{-2} h^{-1} for one month. These values were similar to the *Rh* fluxes (13.5–27.2 mg C m^{-2} h^{-1}) from rice paddies in the Sanjiang Plain of northeast China (Ouyang *et al.*, 2015). Suppression of flooding on *Rs* has also been observed in peatland (McNicol & Silver, 2014) and floodplain (Batson *et al.*, 2015). The O_2 diffusion rate can be decreased by 10,000-fold in wet soil (De-Campos *et al.*, 2012), which is the main cause for decreased *Rh* (Philben *et al.*, 2015). Under anoxic condition, the activities of extracellular oxidative enzymes could be restrained, which has been referred as an “enzymatic latch” mechanism for C store in peatlands (Freeman *et al.*, 2001). In a laboratory experiment, we found that soil microbial biomass measured as total phospholipid fatty acids (PLFAs) content in this soil exhibited a quadratic relationship with WFPS (Fig. S3a). The PLFAs content decreased with increasing soil moisture at > 59% WFPS. Therefore, *Rh* was largely reduced after soil was

waterlogged by the extreme rainfall, although soil DOC concentration tended to increase (Fig. S1c) due to physical disaggregation and organo-mineral complexes destabilization (McNicol & Silver, 2014).

Compared to *Rh*, correlations between *Ra* and rainfall or soil WFPS were less significant (Table S5). The stepwise regression analysis showed that soil temperature and moisture were the main factors controlling *Rh*, while PAR_3 was the only parameter entering the final function for *Ra* (Table 1). Furthermore, annual *Ra* but not *Rh* increased linearly with the aboveground biomass (Fig. 7d). These results suggested that in comparison with *Rh*, *Ra* was less sensitive to the variation in soil moisture (Zimmermann *et al*., 2010; Casals *et al.*, 2011) but more dependent on plant photosynthate supply (Högberg *et al*., 2001; Gomez-Casanovas *et al*., 2012). The entrance of PAR_3 rather than PAR in the regression model indicated a time lag between plant photosynthesis and *Ra* (Kuzyakov & Gavrichkova, 2010).

In the extreme rainfall year of 2013/2014, negative effect of increasing soil moisture on *Ra* occurred at > 60% WFPS (Fig. 6i). During the soil waterlogging period, *Ra* was reduced to less than 15 mg C m^{-2} h^{-1} in both CK and NF treatments. Decreased *Ra* under high soil moisture was mainly due to the restrained activities of roots and rhizosphere microbes by the hypoxia stress (Kreuzwieser & Gessler, 2010; Nottingham *et al*., 2010). Soil waterlogging can rapidly induce stomatal closure to maintain turgor under low leaf water potential and suppress leaf CO_2 assimilation and photosynthetic activity, leading to lower belowground allocation of photosynthates, and thus the decrease of root growth, biomass and exudates (Ashraf & Habib-ur-Rehman, 1999; Milroy & Bange, 2013; Rich & Watt, 2013). After the extreme rainfall, 14 rainfall events occurred in August 2013. During this period, the PAR data was not

available (Fig. 2a), but it could be deduced that PAR should be lower than the corresponding period in normal rainfall years as indicated by the negative relationship between rainfall and PAR ($P < 0.0001$; data not shown). Accordingly, respiration of active roots and rhizospheric microbes would be largely reduced as a result of limited substrates supply (Kuzyakov & Cheng, 2004; Gomez-Casanovas *et al*., 2012).

Based on the decrease of *Rs* after flooding observed under natural conditions, we suggest that a short-term manipulation experiment, with intensive measurement of *Rh* and *Ra* fluxes, should be conducted under water flooding conditions. Dynamics of soil O_2 concentration, microbial biomass, enzyme activities, DOC fraction and plant root biomass are needed to be investigated to get deep insights into mechanisms of *Rs* inhibition by extreme rainfall events.

Effects of extreme snowfall on nongrowing season soil respiration

Most studies on winter C fluxes were conducted in the Arctic, boreal and alpine systems (e.g., Björkman *et al*., 2010; Haei *et al.*, 2011; Wang *et al*., 2014). Our study in the temperate cropland further highlights the significance of cold-season C cycles. *Rs* and *Rh* during the nongrowing season were 15–31 g C m^{-2} and contributed 5.5–16.4% to their annual budget. These values fell within the range of previous records as summarized by Liptzin *et al*. (2009).

Compared with the other two years, nongrowing season *Rs* and *Rh* in 2012/2013 were increased by 28.7% and 26.0%, respectively (Fig. 5). This increment was mainly due to the distinctly higher fluxes during spring thaw (Fig. 2). During the nongrowing season, both *Rs* and *Rh* stemmed from heterotrophic process as no plant roots existed. Elberling & Brandt (2003) indicated that increased CO_2 fluxes after soil freeze-thaw cycles were mainly derived

422 from the physical release of trapped CO_2 in deep layer below the frozen soil. However, we
423 speculated that microbial processes in the thawed soil might be the main source because high
424 CO_2 fluxes appeared when soil had thawed only in the surface (0–30 cm) layer (Monson *et al*.,
425 2006; Wang *et al*., 2013). Soil microbial biomass and activity can be promoted after soil thaw
426 due to the increases in soil temperature, moisture or labile substrates (Liptzin *et al*., 2009;
427 Haei *et al*., 2011). Among these factors, substrate supply has been proposed as the limiting
428 factor for microbial respiration in cold season (Brooks *et al*., 2004; Hirano, 2005). In a
429 laboratory experiment, we found that soil DOC concentration increased, but its specific ultra
430 violet absorption (SUVA, indicator for DOC aromaticity) decreased after a freeze-thaw cycle
431 in test soil at 20–100% WFPS (unpublished data). However, in the field, significant spring
432 thaw CO_2 fluxes were only observed in 2013. During the surface soil thawing period, *Rs* and
433 *Rh* increased more closely with soil moisture than temperature (Fig. S4). WFPS after soil
434 thaw was higher in 2013 than the other two years, while soil temperature was similar among
435 the 3 years (Table S1). Unlike the cases during the growing season, soil microbes were
436 unresponsive to high moisture condition after exposure to freeze-thaw (Fig. S3). Accordingly,
437 we speculated that higher soil moisture content was likely the main contributor for higher *Rs*
438 and *Rh* during the spring thaw period in 2013. More frequent snowfall and especially the
439 extreme snowfall event in winter of 2012/2013 resulted in more total snowfall and deeper
440 snow cover than 2011/2012 and 2013/2014 (Table S1). More melting water induced higher
441 soil moisture content (Maurer & Bowling, 2014) and thus higher CO_2 fluxes in spring 2013.
442 In accordance with our findings, Nobrega & Grogan (2007) observed higher *Rs* during the
443 spring thaw period in the snow fence treatment with deeper snow cover. Björkman *et al*.

(2010) also reported an exponential relationship between snow depth and winter soil CO_2 fluxes in Arctic ecosystems.

The occurrence of extreme snowfall event is predicted to intensify in the Northern Hemisphere under warming climate (O'Gorman, 2014). Moreover, higher Q_{10} values of *Rs* and *Rh* during the nongrowing season (Table S6) indicated greater sensitivity of respiration to climate warming at lower temperature, especially under wetter condition (Davidson & Janssens, 2006; Wang *et al*., 2014). Thus, it is crucial to pay more attention on cold season respiration and its responses to climate warming.

Effects of N fertilization and its responses to extreme precipitation

N fertilization increased *Rs* by 23.9% and 10.9% in the normal rainfall years of 2011/2012 and 2012/2013, respectively. Similarly, with a meta-analysis, Zhou *et al*. (2014) found that N addition enhanced *Rs* by 12.4% for the cropland ecosystems. Stimulated *Rs* by N fertilization in the normal years was mainly due to the positive response of *Ra*, which was increased by 38.0% and 31.9% in 2011/2012 and 2012/2013, respectively. On the other hand, no significant difference in *Rh* was observed between CK and NF in each year. Likewise, Hasselquist *et al*. (2012) reported that N addition increased *Rs* as a result of elevated *Ra* in a boreal forest. We found that *Ra* increased linearly with increase in the aboveground biomass (Fig. 7d), which was higher in NF than CK in 2011/2012 and 2012/2013 (Table S7). Consistently, Yan *et al*. (2010) showed that N addition increased *Ra* as a result of increased plant productivity in a temperate steppe. Zhang *et al*. (2014) proposed that N addition improved root growth and N accumulation in root tissues and thus facilitated root respiration, which was speculatively also

466 the case in the normal rainfall years of our studies.

467 In the record wet year of 2013/2014, *Rs* was almost equal between CK and NF (Fig. 4).
468 Based on the synthetic data, we found that the effect of N fertilization on *Rs* could be well
469 fitted to precipitation by a Gaussian function with an optimal precipitation of 531 mm during
470 the maize growing season in the study region (Fig. 8b). These previous studies did not
471 partition respiration component. In the present study, we found that the neutral effect of N
472 fertilization on *Rs* under excessively wet condition was mainly due to the inhibition of *Ra*.
473 After soil was waterlogged by the extreme rainfall, *Ra* was decreased to no more than 15 mg
474 C m^{-2} h^{-1} in both CK and NF treatments lasting for about one month. Limited photosynthate
475 supply as a result of inhibited plant photosynthesis was suggested as the main cause for
476 decreased Ra as discussed above. Ashraf & Habib-ur-Rehman (1999) reported that the
477 decreases of chlorophylls content and substomatal CO_2 concentration in maize leaves induced
478 by waterlogging were greater under higher rate of N fertilization. They pointed out that
479 increased N supply could have injurious effects on the growth of maize experiencing soil
480 waterlogging. In the current study, we found that, in contrast with the other years, the plant
481 biomass in CK was higher, albeit not significantly, than in NF in 2013/2014 (Table S7). As a
482 consequence, the positive effects of N fertilization on *Ra* and accordingly *Rs* disappeared in
483 the extreme rainfall year.

484 No significant effect of N fertilization on annual *Rh* was found in each year, regardless of
485 the occurrence of extreme precipitation event. Interestingly, the temporal variation of the RR
486 of *Rh* flux was consistent among the 3 years (Fig. 3). Initial positive effect of N fertilization
487 on *Rh* was probably because of the stimulated decomposition of labile SOM compounds. It

has been suggested that N addition could enhance the activities of hydrolytic enzymes that primarily decompose the labile organic matter (Knorr *et al*., 2005) and the competitive ability of r-strategist microbes that are more N-demanding (mainly bacteria) under sufficient C supply (Ågren *et al*., 2001). As the labile substrates were gradually consumed, the RR of *Rh* became lower and turned to below zero. At this time, a higher proportion of heterotrophic CO_2 fluxes were likely sourced from the fungal decay of more recalcitrant organic matter (Knorr *et al*., 2005; Berg, 2014), which could be inhibited by N addition (Treseder, 2008; Xu *et al*., 2016). During the nongrowing season, *Rh* in NF tended to be higher again than in CK. Nutrients availabilities are suggested to be low and play a key role for microbes maintaining metabolically activity in frozen soils (Lukas *et al*., 2013). N addition could alleviate nutrient limitation and thus promote microbial respiration. Our laboratory experiment showed that the concentration and decomposability of soil DOC as well as microbial biomass were increased after thaw in test soil (unpublished data) as supported by Haei *et al*. (2011). Consequently, peak value of the RR of *Rh* was found during the spring thaw period in each year.

The RR of nongrowing season cumulative *Rs* and *Rh* was greater in the normal year of 2011/2012 than the year of 2012/2013 and 2013/2014 with extreme rainfall or snowfall events (Fig. 5). The impact of soil moisture on CO_2 fluxes during the spring thaw period was the main reason causing the interannual variations of nongrowing season *Rs* and *Rh* as discussed above. The RR of *Rs* and *Rh* decreased with increasing soil WFPS during the spring thaw period across the 3 years (Fig. S5). Higher soil moisture condition is beneficial for microbial nutrient acquisition and thus alleviates the restriction of nutrients on microbial activities in cold environment (Lukas *et al*., 2013). Compared with the spring thaw period in 2011/2012,

soil moisture was higher in 2012/2013 and 2013/2014 (Fig. 2b) due to more snow melting water and the legacy effect of the extreme rainfall in summer 2013 (Chen *et al*., 2016), respectively. Accordingly, *Rs* and *Rh* was less responsive to N fertilization during the nongrowing season of 2012/2013 and 2013/2014 than 2011/2012.

In summary, our 3-year field experiment showed that *Rs* was stimulated by N fertilization in normal rainfall years as a result of the increase in *Ra*. While, *Rh* was unresponsive to N fertilization. However, in the record wet year, there was no significant effect of N fertilization on *Rs* due to the inhibited effects of an extreme summer rainfall event on *Rh* and particularly *Ra*. Reduced *Rh* and *Ra* were mainly attributable to the anoxic stress on soil microorganisms and decreased plant photosynthesis, respectively. Nongrowing season *Rs* and *Rh* played an important role in their annual budgets, and were increased by the extreme snowfall event due to high soil moisture content during spring thaw. The positive effect of N fertilization on nongrowing season *Rs* and *Rh* was weaker in the years with extreme snowfall or rainfall than normal year. These findings have important implications on soil C flux and its feedback to climate change because extreme rainfall and snowfall will be more frequent and intense with warming climate in the Northern Hemisphere, especially in mid-high latitudes (Fischer *et al*., 2013, O'Gorman, 2014). Reduced *Rs* and its response to N supply by extreme rainfall may generate a negative feedback to climate change. However, considering higher temperature sensitivity of *Rs* at lower temperature (Carey *et al*., 2016), increased nongrowing season *Rs* under extreme snowfall regimes would have a large potential for causing positive climate change feedback. Therefore, the effects of extreme precipitation on soil C cycling and its response to N fertilization should be incorporated into earth system models to improve the

prediction of C-climate feedbacks. However, cautions should be taken when extrapolating our results to other regions. Cross-site and cross-biome studies are necessary to confirm our findings at a large scale. Manipulation studies, combining a wide gradient of precipitation and N addition rate and focusing on soil microbial processes and plant physiology, are needed for better quantitative and mechanistic understanding on the responses of soil C flux to N enrichment under extreme precipitation events.

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771 climate dependence of soil respiration and its components along a 3000 m altitudinal
772 tropical forest gradient. *Global Biogeochemical Cycles*, **24**, GB4012.

773 **Supporting Information**

774 **Table S1** Weather parameters over a 3-year period from May 2011 to April 2014.

775 **Table S2** Results of the repeated-measures ANOVA analysis of the effects of sampling time,
776 treatment and their interaction on the fluxes of soil respiration (*Rs*), heterotrophic (*Rh*) and
777 autotrophic respiration (*Ra*) in 2011/2012, 2012/2013 and 2013/2014.

778 **Table S3** Correlations between soil respiration and total precipitation over 3 days prior to gas
779 measurement (Pr), photosynthetically active radiation (PAR), average PAR (PAR_3) over 3
780 days prior to gas measurement, soil temperature (ST), water-filled pore space (WFPS),
781 ammonium (NH_4^+), nitrate (NO_3^-) or dissolved organic carbon (DOC) concentrations.

782 **Table S4** Correlations between heterotrophic respiration and total precipitation over 3 days
783 prior to gas measurement (Pr), photosynthetically active radiation (PAR), average PAR over 3
784 days prior to gas measurement (PAR_3), soil temperature (ST), water-filled pore space (WFPS),
785 ammonium (NH_4^+), nitrate (NO_3^-) or dissolved organic carbon (DOC) concentrations.

786 **Table S5** Correlations between autotrophic respiration and total precipitation over 3 days
787 prior to gas measurement (Pr), photosynthetically active radiation (PAR), average PAR over 3
788 days prior to gas measurement (PAR_3), soil temperature (ST), water-filled pore space (WFPS),
789 ammonium (NH_4^+), nitrate (NO_3^-) or dissolved organic carbon (DOC) concentrations.

790 **Table S6** Temperature sensitivity (Q_{10} value) of soil respiration (*Rs*), heterotrophic (*Rh*) and
791 autotrophic (*Ra*) respiration during the growing and nongrowing seasons and the whole year
792 in 2011/2012, 2012/2013, 2013/2014 and across the three years.

793 **Table S7** Soil organic carbon (SOC), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-)
794 and dissolved organic carbon (DOC) concentrations at the end of experiment and plant

795 biomass in the experimental year of 2011/2012, 2012/2013 and 2013/2014.

796 **Fig. S1** Soil ammonium (NH_4^+; a), nitrate (NO_3^-; b) and dissolved organic carbon (DOC; c)
797 concentrations in the treatment without (CK) and with (NF) nitrogen fertilization over a
798 3-year measurement period from May 2011 to April 2014. Soil DOC concentrations were not
799 determined in 2011/2012. Vertical bars are the standard errors of the means. Arrows denote
800 the fertilization time. Shading parts represent the nongrowing season period.

801 **Fig. S2** Autotrophic respiration (*Ra*) from soils without (CK) and with (NF) nitrogen
802 fertilization in each measurement year from 2011 to 2013. Vertical bars are the standard errors.
803 Arrows denote the fertilization time.

804 **Fig. S3** Relationship between total phospholipid fatty acids (PLFAs) in test soil and
805 water-filled pore space (WFPS) in laboratory incubation experiments. Data in the right panel
806 were measured after a freeze-thaw cycle (soils were frozen at −25 °C for 7 days and then
807 thawed at 8 °C). Vertical bars are the standard errors (n = 4).

808 **Fig. S4** Relationships between soil respiration (*Rs*; a, b) or heterotrophic respiration (*Rh*; c, d)
809 and soil water-filled pore space (WFPS) or temperature during the surface (0–30 cm) soil
810 thawing period over the three measurement years. Dashed lines indicate the bounds of the 95%
811 confidence intervals.

812 **Fig. S5** Relationships between soil water-filled pore space (WFPS) and the response ratio of
813 soil respiration (*Rs*) or heterotrophic respiration (*Rh*) to nitrogen fertilization during the spring
814 thaw period over the three measurement years.

Table 1 Stepwise regression analysis of soil respiration (*Rs*), heterotrophic (*Rh*) and autotrophic (*Ra*) respiration with photosynthetically active radiation (PAR), mean PAR over 3 days prior to gas measurement (PAR_3), soil temperature (ST), water-filled pore space (WFPS), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations across the three years.

		Equation	*n*	*F*	R^2	*P*
CK	*Rs*	$Y = 2.79\ PAR_3$	53	203	0.80	< 0.0001
		$Y = 3.29\ PAR_3 - 1.85\ NO_3^-$	53	111	0.81	< 0.0001
		$Y = 1.95\ PAR_3 - 2.06\ NO_3^- + 2.13\ ST$	53	82	0.83	< 0.0001
	Rh	$Y = 2.10\ ST$	53	281	0.84	< 0.0001
		$Y = 1.44\ ST + 0.31\ WFPS$	53	162	0.86	< 0.0001
		$Y = 1.63\ ST + 0.36\ WFPS - 0.78\ NO_3^-$	53	118	0.88	< 0.0001
	Ra	$Y = 1.34\ PAR_3$	53	98	0.65	< 0.0001
		$Y = 1.68\ PAR_3 - 1.30\ NO_3^-$	53	55	0.68	< 0.0001
		$Y = 0.86\ PAR_3 - 1.70\ NO_3^- + 0.95\ PAR$	53	42	0.72	< 0.0001
NF	*Rs*	$Y = 4.36\ ST$	53	227	0.81	< 0.0001
		$Y = 3.29\ ST + 8.38\ NH_4^+$	53	123	0.83	< 0.0001
	Rh	$Y = 2.02\ ST$	53	447	0.90	< 0.0001
		$Y = 1.37\ ST + 0.30\ WFPS$	53	288	0.92	< 0.0001
	Ra	$Y = 1.69\ PAR_3$	53	112	0.68	< 0.0001
All	*Rs*	$Y = 4.21\ ST$	106	423	0.80	< 0.0001
		$Y = 2.26\ ST + 1.40\ PAR_3$	106	231	0.82	< 0.0001
	Rh	$Y = 2.06\ ST$	106	688	0.87	< 0.0001
		$Y = 1.40\ ST + 0.31\ WFPS$	106	417	0.89	< 0.0001
	Ra	$Y = 1.52\ PAR_3$	106	206	0.66	< 0.0001

819 **Figure legends**

820 **Fig. 1** Scatter plots of total precipitation and mean air temperature for the whole year (a) and
821 winter period (November to March) (b) of the study region from 1953 to 2013. Solid squares
822 indicate the measurement years. Dashed lines represent the 60-year averages.

823

824 **Fig. 2** Air (AT) and soil temperature (ST), photosynthetically active radiation (PAR), snow
825 depth (a), precipitation, soil water-filled pore space (WFPS; b), soil respiration (*Rs*; c) and
826 heterotrophic respiration (*Rh*; d) from soils without (CK) and with (NF) nitrogen fertilization
827 from May 2011 to April 2014. Vertical bars are the standard errors of the means. Arrows
828 denote the fertilization time. Shading parts represent the nongrowing season.

829

830 **Fig. 3** Temporal variation of the response ratio (RR) of the flux of autotrophic (*Ra*) or
831 heterotrophic (*Rh*) respiration to nitrogen fertilization in 2011/2012 (a), 2012/2013 (b) and
832 2013/2014 (c). Relationship between RR of *Rh* and experimental day was fitted with a cubic
833 model in each year. Dashed lines indicate the bounds of the 95% confidence intervals.

834

835 **Fig. 4** Annual soil respiration (*Rs*; a), heterotrophic respiration (*Rh*; b), autotrophic respiration
836 (*Ra*; c), and the contribution of annual *Rh* to *Rs* (d) for the experimental year of 2011/2012,
837 2012/2013 and 2013/2014 and across the three years. Values are means ± SE. Significant
838 differences between treatments and years at $P < 0.05$ are indicated by different lowercase and
839 capital letters, respectively.

840

841 **Fig. 5** Nongrowing season soil respiration (*Rs*; a), heterotrophic respiration (*Rh*; b), and their
842 response ratio (RR) to nitrogen fertilization for the experimental year of 2011/2012,
843 2012/2013 and 2013/2014 and across the three years. Values are means ± SE. Significant
844 differences between treatments and years at $P < 0.05$ are indicated by different lowercase and
845 capital letters, respectively.
846
847 **Fig. 6** Relationships between soil water-filled pore space (WFPS) and soil respiration (*Rs*),
848 heterotrophic (*Rh*) or autotrophic (*Ra*) respiration during the growing season of 2011/2012 (a,
849 d and g), 2012/2013 (b, e and h) and 2013/2014 (c, f and i).
850
851 **Fig. 7** Relationships between the annual soil respiration (*Rs*), heterotrophic (*Rh*) or
852 autotrophic (*Ra*) respiration and annual mean soil ammonium (NH_4^+; a), nitrate (NO_3^-; b) and
853 dissolved organic carbon (DOC; c) concentrations and total aboveground biomass (d).
854
855 **Fig. 8** Relationships between precipitation and soil respiration (*Rs*) from the N-unfertilized
856 (CK) and N-fertilized (NF) black soils (a) or the response ratio (RR) of *Rs* to N fertilization (b)
857 during the maize growing season. The shading part represents the 95% confidence intervals.
858 Data were sourced from the present study and previous studies conducted in the same region
859 as ours (Li *et al*., 2009; Liang *et al*., 2012; Ni *et al*., 2012; Li *et al*., 2013). In the up panel,
860 data in the year of 2013/2014 with the extreme rainfall events in the present study were not
861 included in the regression analysis, and the open square and dots represent data calculated
862 based on the linear function and precipitation during the growing season in 2013/2014.

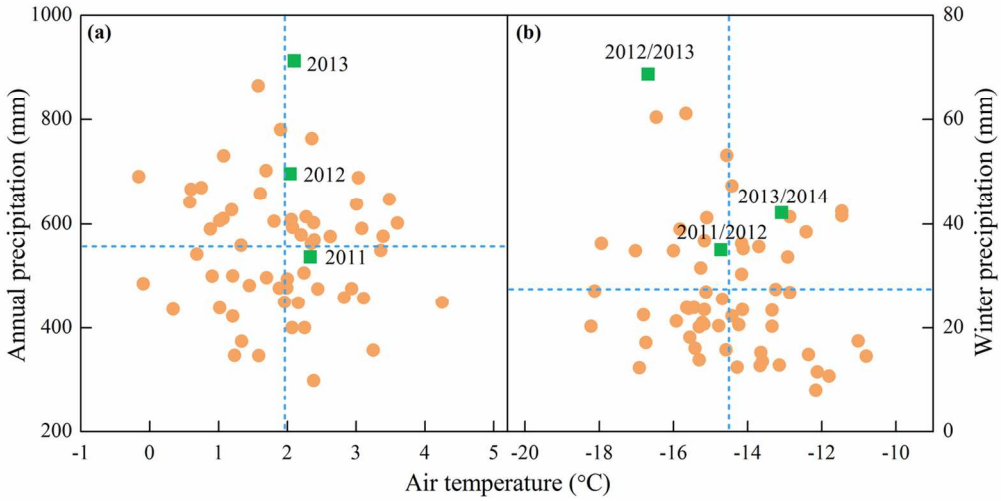

Fig. 1 Scatter plots of total precipitation and mean air temperature for the whole year (a) and winter period (November to March) (b) of the study region from 1953 to 2013. Solid squares indicate the measurement years. Dashed lines represent the 60-year averages.

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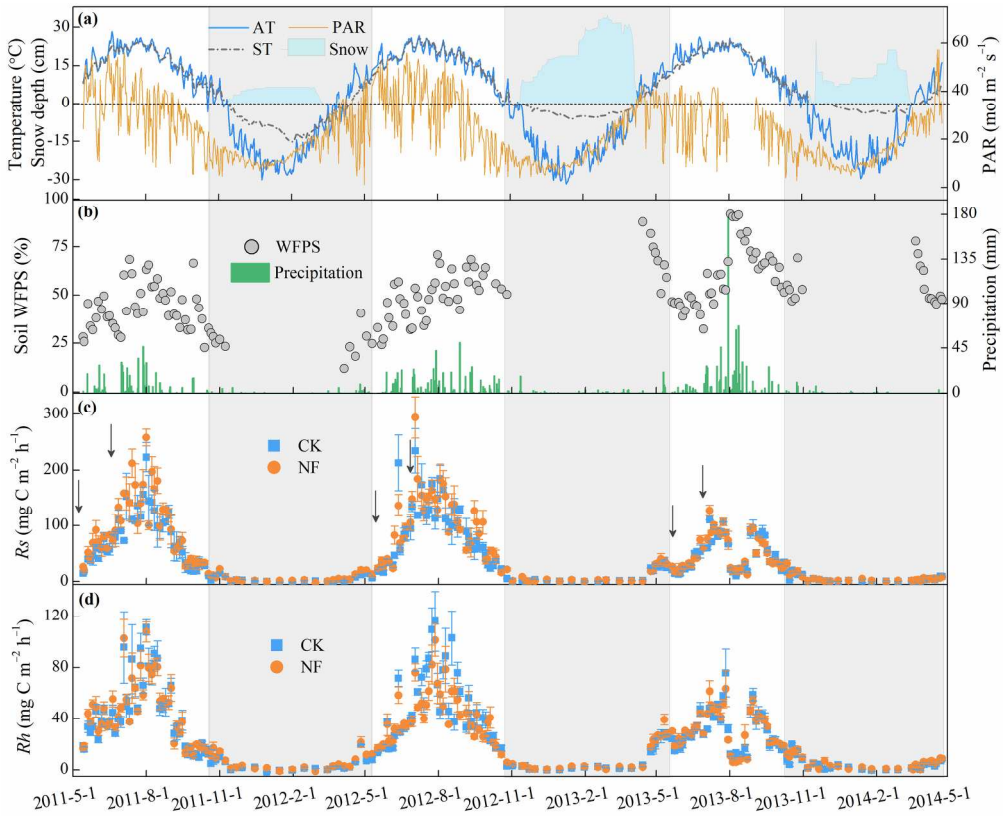

Fig. 2 Air (AT) and soil temperature (ST), photosynthetically active radiation (PAR), snow depth (a), precipitation, soil water-filled pore space (WFPS; b), soil respiration (Rs; c) and heterotrophic respiration (Rh; d) from soils without (CK) and with (NF) nitrogen fertilization from May 2011 to April 2014. Vertical bars are the standard errors of the means. Arrows denote the fertilization time. Shading parts represent the nongrowing season.

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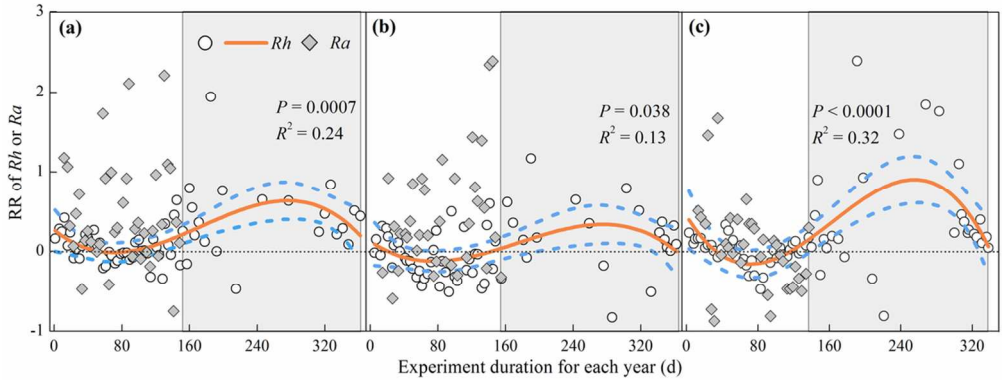

Fig. 3 Temporal variation of the response ratio (RR) of the flux of autotrophic (Ra) or heterotrophic (Rh) respiration to nitrogen fertilization in 2011/2012 (a), 2012/2013 (b) and 2013/2014 (c). Relationship between RR of Rh and experimental day was fitted with a cubic model in each year. Dashed lines indicate the bounds of the 95% confidence intervals.

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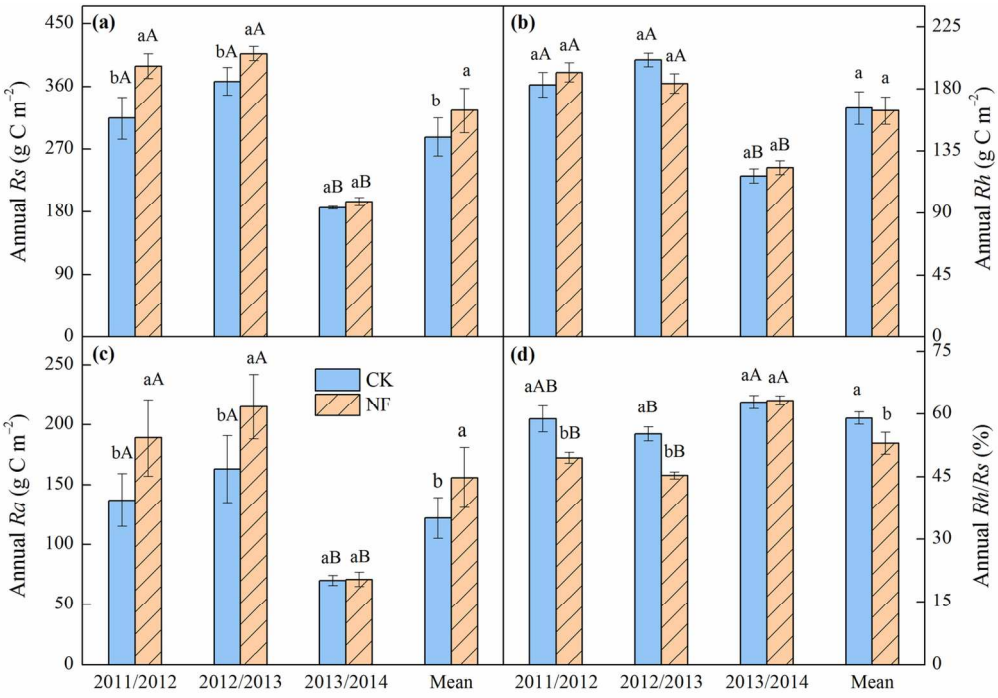

Fig. 4 Annual soil respiration (Rs; a), heterotrophic respiration (Rh; b), autotrophic respiration (Ra; c), and the contribution of annual Rh to Rs (d) for the experimental year of 2011/2012, 2012/2013 and 2013/2014 and across the three years. Values are means ± SE. Significant differences between treatments and years at $P < 0.05$ are indicated by different lowercase and capital letters, respectively.

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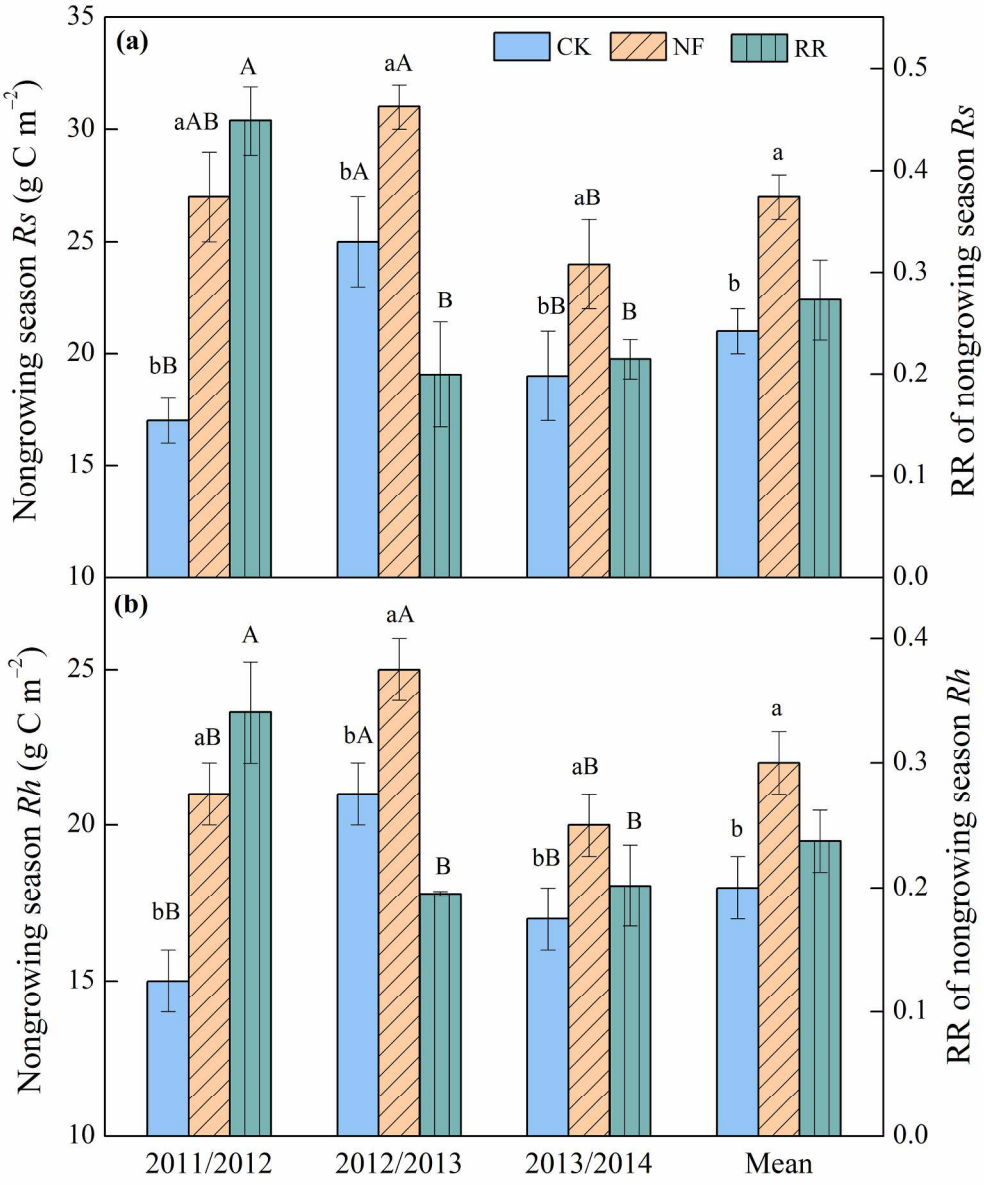

Fig. 5 Nongrowing season soil respiration (Rs; a), heterotrophic respiration (Rh; b), and their response ratio (RR) to nitrogen fertilization for the experimental year of 2011/2012, 2012/2013 and 2013/2014 and across the three years. Values are means ± SE. Significant differences between treatments and years at $P < 0.05$ are indicated by different lowercase and capital letters, respectively.

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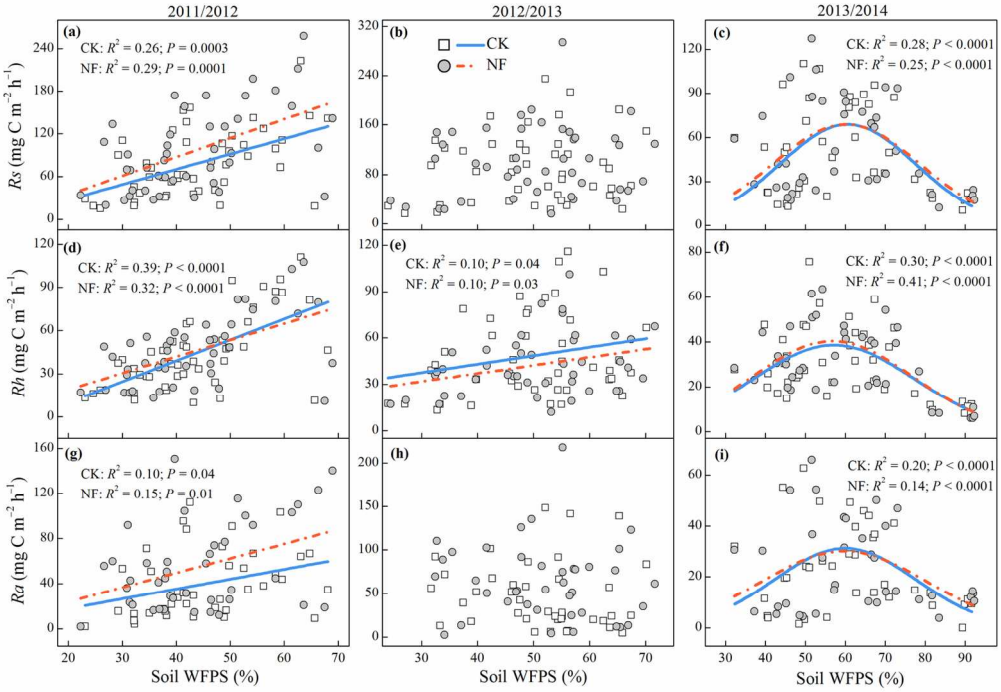

Fig. 6 Relationships between soil water-filled pore space (WFPS) and soil respiration (Rs), heterotrophic (Rh) or autotrophic (Ra) respiration during the growing season of 2011/2012 (a, d and g), 2012/2013 (b, e and h) and 2013/2014 (c, f and i).

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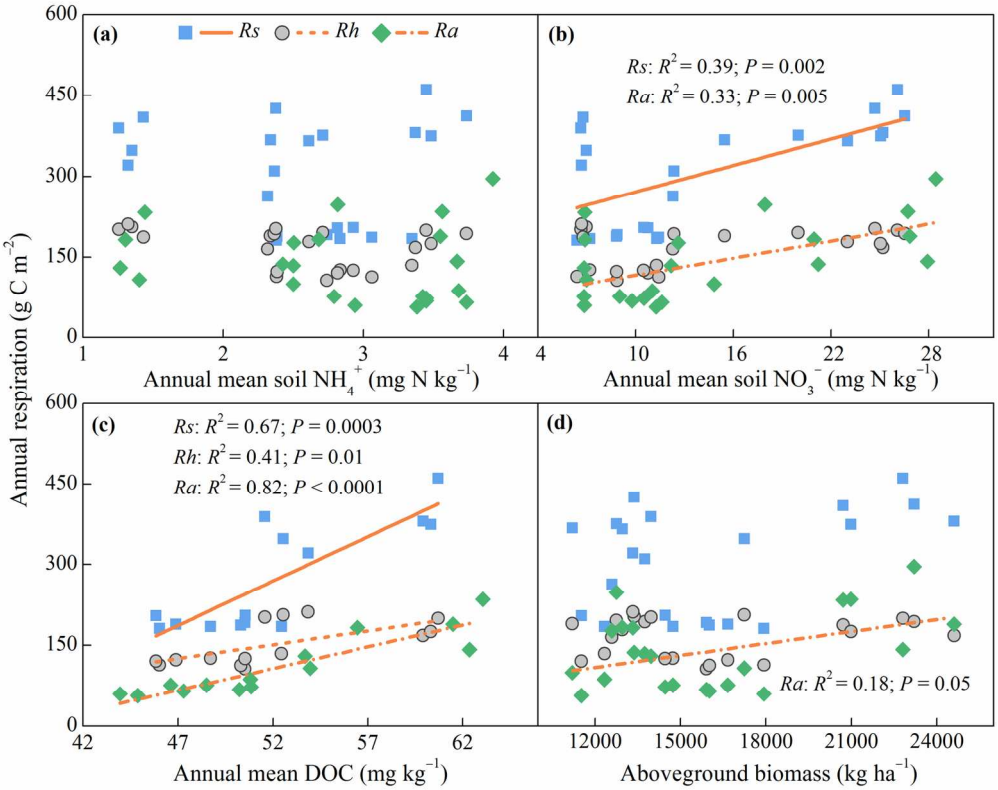

Fig. 7 Relationships between the annual soil respiration (Rs), heterotrophic (Rh) or autotrophic (Ra) respiration and annual mean soil ammonium (NH4+; a), nitrate (NO3−; b) and dissolved organic carbon (DOC; c) concentrations and total aboveground biomass (d).

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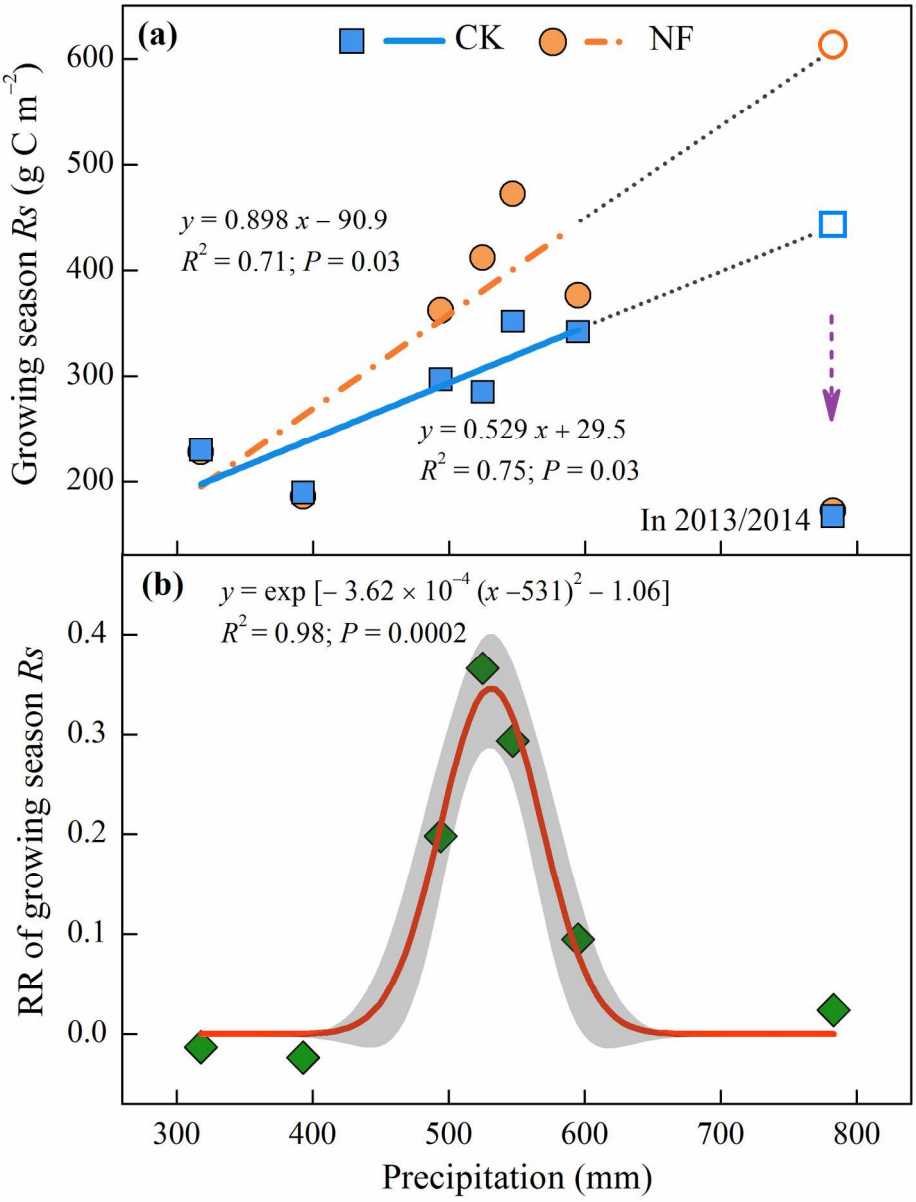

Fig. 8 Relationships between precipitation and soil respiration (Rs) from the N-unfertilized (CK) and N-fertilized (NF) black soils (a) or the response ratio (RR) of Rs to N fertilization (b) during the maize growing season. The shading part represents the 95% confidence intervals. Data were sourced from the present study and previous studies conducted in the same region as ours (Li et al., 2009; Liang et al., 2012; Ni et al., 2012; Li et al., 2013). In the up panel, data in the year of 2013/2014 with the extreme rainfall events in the present study were not included in the regression analysis, and the open square and dots represent data calculated based on the linear function and precipitation during the growing season in 2013/2014.

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